Novel Benzoxazolylidene Probes for the Imaging of Apoptosis by PET and SPECT.

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Abstract

Agents for the in vivo detection of apoptosis were developed based on the structure of Yo-Pro III, a commercially available di-cationic benzothiazole DNA dye. Four analogs were synthesized containing nonradioactive isotopes of positron- or gamma-emitting isotopes commonly used in medical imaging: C, **5**; Br, **6**; I, **7**; and F, **9**. The four were tested to determine if these compounds maintained their binding affinity for DNA after the modification of their structure, and spectral data indicated a strong attraction to DNA was retained for all four compounds. In addition, cell culture studies performed with radiation-induced apoptotic cells showed that the analogs maintained both their high level of uptake and selectivity for apoptotic over normal cells.

Introduction

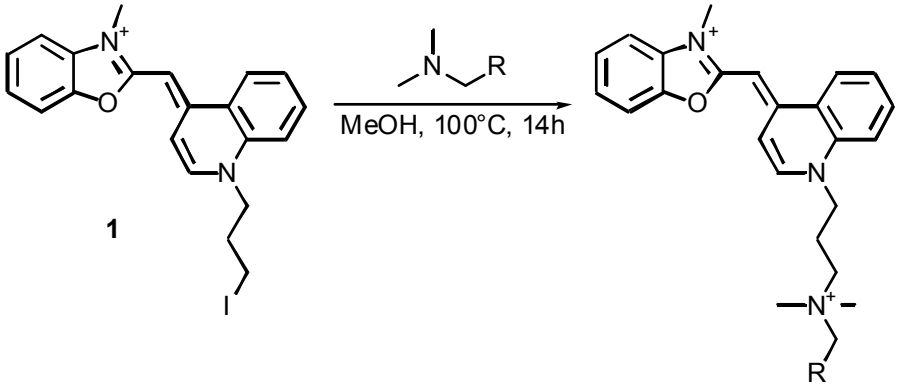
The development of a radioligand that would target an early cellular response to radiation or chemotherapy could facilitate the establishment of a medical imaging protocol to allow clinicians to make an immediate assessment of the success of a given therapeutic approach. One cellular process has already been identified and targeted: apoptosis. Apoptosis is an organized sequence of controlled catabolic events that results in cell death with limited collateral damage (Ashkenazi and Dixit 1998). Apoptosis is one mechanism of tumor cell death following therapy (LaCasse et. Al 1998). Accordingly, apoptosis in tumors has become the target for imaging agents (Blankenberg 1999). Knowing if apoptosis has been induced within the tumors early in the course of therapy will allow for refinement of therapeutic selection and scheduling during agent development and could be used to select patient specific therapies or to limit patient exposure to therapy regimes that are not inducing the desired response.

Fluorescent apoptotic cell markers have been developed that take advantage of the disruption of cell membrane integrity that occurs during apoptosis to differentiate between apoptotic and normal cells (Idziorek et al. 1995). These dyes bind strongly to all cellular DNA, but being cationic cannot penetrate the cellular membrane until apoptosis has been initiated. Only after binding to the DNA do these dyes become fluorescently active.

A similar approach for the nuclear imaging of apoptosis has been developed using analogs to one of these di-cationic fluorescent dyes that can be synthesized with radioisotopes. They will selectively image apoptosis since they can only penetrate and be retained in apoptotic cells. The development of nonradioactive analogs of the benzothiazole di-cationic apoptosis marker Yo-Pro (Molecular Probes) is described below. In future studies, radiolabeled versions of these analogs will be produced to facilitate the imaging of tumor apoptosis following chemo- and radiotherapy with either positron emission tomography (PET) or single photon emission computed tomography (SPECT).

Chemistry

The general route to cyanines **5-7** and **9** is described in scheme 1. Iodopropyl oxazole **1**, prepared by the methodology described by Rye et al,[1] was reacted with tertiary amines as a methanolic solution in a sealed reaction vessel for 14 hours at 100°C. Upon cooling, the dicationic products precipitated out of solution and were recrystallised from methanol to give pure product.



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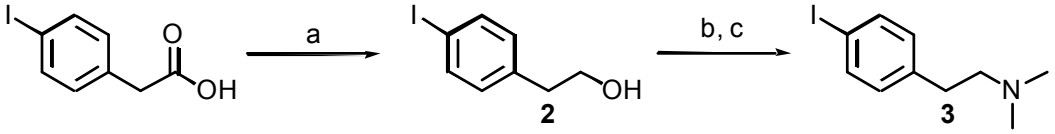
Scheme 2 describes the synthesis of 4-iodophenylethyl-N,N-dimethylamine. Commercially available 4-iodobenzene carboxylic acid was reduced to alcohol **2** using lithium aluminum hydride. Tosylation with tosyl chloride and trimethylamine followed by amination with dimethylamine gave tertiary amine **3**.

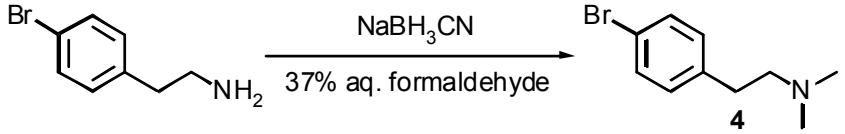


2 **3**

Reagents and conditions; a) $LiAlH_4$, Et_2O, 0°C to RT, 14h; b) TsCl, Et_3N, CH_2Cl_2, 0°C to RT, 14h; c) NMe_2 (2M in Methanol), sealed reaction vessel, 60°C, 6h.

Scheme 2

4-Bromo tertiary aniline **4** was prepared as described in scheme 3. Commercially available 4-bromophenethylamine was bis-methylated using aqueous formaldehyde in the presence of sodiumcyanoborohydride as described by Borch et al[3].



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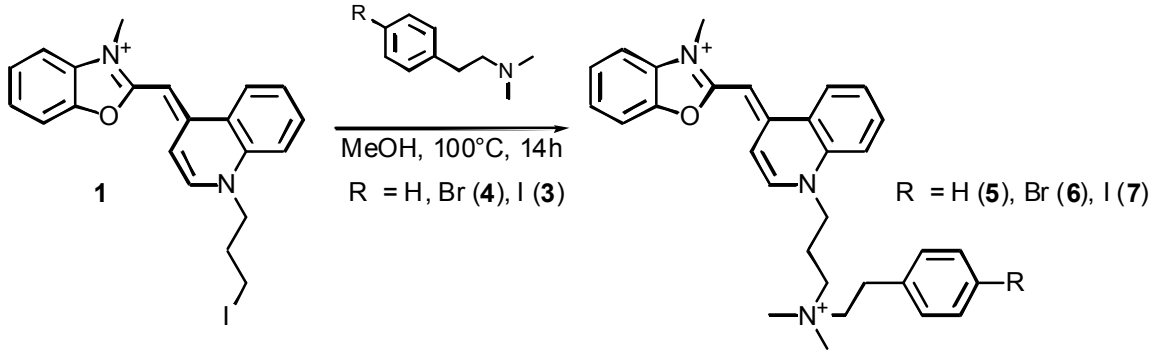
Scheme 3

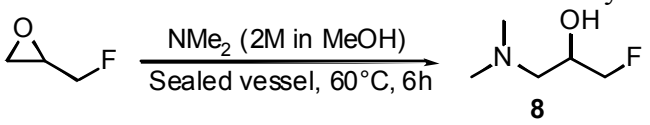
Tertiary amines **3** and **4** along with commercially available N,N-dimethylphenethylamine were reacted with oxazole **1** (scheme 4) to give dicationioc cyanines **5-7**, potentially

useful as SPECT imaging agents via radio-labeling of the appropriate tertiary amines with 11-carbon, 76-bromine or 123-iodine.



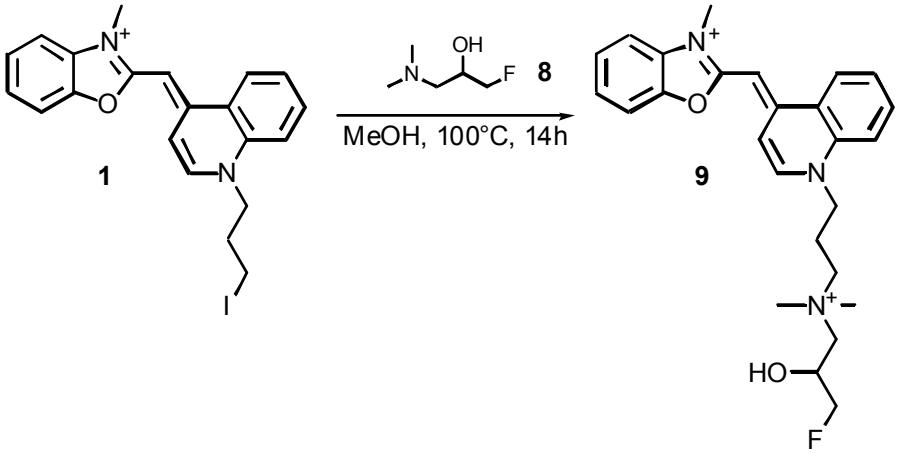
Scheme 4

Scheme 5 describes the synthesis of fluorine containing tertiary amine for the potential use in PET imaging. Opening of epifluorohydrin by dimethylamine in a sealed reaction vessel gave tertiary amine **8** in a similar fashion to that described by Cherbuliez et al[4].



Scheme 5

Tertiary amine **8** was reacted in the same manner as described previously to give the potential PET imaging agent, cyanine **9**, via radio-labeled 18-fluorine epifluorohydrin.



Scheme 6

Biological Data

The difference in uptake across the cellular membrane between apoptotic and non-apoptotic cells will be the critical factor in the success of these compounds as imaging agents. An additional factor could be the further retention of these agents within the apoptotic cells due to their interaction with cellular DNA, allowing for clearance of the imaging agents from other tissues while the label is retained in the target cells, leading to signal enhancement in the apoptotic cells versus that in non-responding tissue. Thus it is important to know if the modifications made to the analogs inhibit binding to DNA.

We found that the modifications did not greatly alter the fluorescent properties of the compounds, so changes in absorbance maxima and fluorescent yield in the presence and absence of DNA could be used as an indicator of DNA binding by these dyes. As shown in Table 1**,** all four compounds were found to be fluorescently inert in the absence of DNA, but showed a shift in absorbance maximum and a strong fluorescent signal with the addition of calf thymus DNA, evidence that the modifications did not alter the ability of the analogs to bind to DNA.

Table 1 Spectral Characteristics of Analogs in the Presence of DNA

Analog	Absorbance max w/o DNA	Emission max w/o DNA	Absorbance max + DNA	Emission max + DNA
5	480 nm	None detected	490 nm	508 nm
6	481 nm	None detected	491 nm	508 nm
7	480 nm	None detected	491 nm	506 nm
9	481 nm	None detected	491 nm	507 nm
Yo-Pro	480 nm	None detected	490 nm	510 nm

Another potentially detrimental outcome of the chemical modifications could be an alteration in their uptake across the plasma membrane, resulting in either enhanced uptake into non-apoptotic cells or reduced uptake into apoptotic cells, either of which would reduced the difference in signal between apoptotic tumor cells and the surrounding tissue. The occurrence of such an outcome was tested using a tissue culture system with apoptosis induced by radiation. Apoptotic cells were imaged by fluorescent microscopy and counted based on their cellular uptake, as indicated by fluorescent signal. As illustrated in Table II, all four analogs in question showed marked differences in the frequency of apoptotic cells between irradiated and sham-irradiated cultures, and were nearly identical to results obtained with the commercially available Yo-Pro dye. In addition, the modifications in chemical structure did not result in enhanced uptake into the normal cells, so not only were the compounds still able to pass through apoptotic membranes, but any structurally-induced changes in lipophilicity did not increase non-specific uptake into normal cells. Increased non-specific binding to the outer membrane or internal cellular structures could not be ruled out with these assays that require binding to DNA to be measured; this will be determined in the future with radiolabeled analogs.

Table II Uptake of Analogs into Apoptotic and Normal Cells

Analog	Number of fluorescent cells following irradiation	Number of fluorescent cells following sham-irradiation
5	33.0 ± 7.5	3.3 ± 2.5
6	38.0 ± 7.2	2.7 ± 1.2
7	35.7 ± 9.0	3.7 ± 0.6
9	35.7 ± 5.8	3.7 ± 0.6

Yo-Pro	33.7 ± 2.1	2.3 ± 1.2

n=3

Conclusions

Four analogs to the commercially available benzothiazole di-cationic apoptosis marker dyes were successfully produced. All four of these compounds retained their affinity for DNA and their selectivity in crossing the membrane of apoptotic cells. These compounds represent nonradioactive versions of compounds that will be labeled with radioisotopes (**5**, 11-C; **6**, 76-Br; **7**, 123-I, 124-I; **9**; 18-F) for use in PET- or SPECT-based imaging of therapy-induced tumor apoptosis to assist in determining the best course of action of cancer therapy.

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Experimental

General

All chemicals and solvents were obtained from Aldrich Chemical Co. (Milwaukee, WI), apart from fluorochloromethane, which was purchased from Synquest Labs (Alachua, FL) and were used without further purification. All reactions were performed under an argon atmosphere. All NMR spectra were recorded using either a Bruker VBAMX 400 or AMX 300 MHz. HPLC was carried out an analytical Whatman ODS 3 C-18 column (4.6 x 250 mm) using a Waters 600E Multisolvent delivery system with in-line UV monitoring of the effluent at 254 nm. Low and high resolution mass spectra (FAB, EI, CI) were completed at the Mass Spectrometry Laboratory of the Department of Chemistry at University of California, Berkeley. Some low resolution mass spectra were completed using a Thermquest Finnigan LCQ™-Duo Mass Spectrometer (ESI, APCI) and processed with Xcalibur™ data system.

2-Methylthiobenzoxazole

2-Mercaptobenzoxazole (1.0 g, 6.61 mmol), acetone (14 ml), anhydrous potassium carbonate (1.01 g, 7.28 mmol), and iodomethane (0.45 ml, 7.28 mmol) were added sequentially to a round bottom flask. The mixture was stirred for 5 h at room temperature and was subsequently filtered through Celite to remove the potassium salts. Acetone was removed by reduced pressure and the isolated oil was dried by high vacuum to give colorless oil in quantitative yield (1.09 g, 100%). ^{1}H-NMR (400 MHz, CD_3Cl) ∂ 2.74 (s, 3H), 7.58 (m, 2H) and 7.74 (m, 2H).

2-Methylthio-N-methylbenzoxazolium triflate

Methyl trifluoromethanesulfonate (4.17 ml, 36.86 mmol) was added to a conical vial containing 2-methylthiobenzoxazole (3.0 g, 18.16 mmol) and a spin vane. The reaction was sealed, heated to approximately 60 °C, and stirred overnight. Once cooled to room temperature, the isolated white precipitate was washed copiously with diethyl ether. The crude product was crystallized from 10% dichloromethane/ethyl acetate to give white needles in nearly quantitative yield (**3**, 5.82 g, 97%). ^{1}H-NMR (400 MHz, $CDCl_3$) ∂ 3.03 (s, 3H), 3.91 (s, 3H), 7.58 (m, 2H) and 7.74 (m, 2H).

Lepidine iodopropiodide

1,3-Diiodopropane (6.02 ml, 52.38 mmol) was added to a 10ml conical vial containing lepidine (2.77 ml, 20.95 mmol) and a spin vane. The reaction was sealed, heated to 130 °C, and stirred until mixture became a yellow solid (approximately 2 h). The crude product was crystallized from 5% methanol/ethyl acetate to give yellow needles (8.46 g, 92%): ^{1}H-NMR (300 MHz, $CDCl_3$) ∂ 2.68 (m, 2H, J = 6.75, 7.63 Hz), 3.11 (s, 3H), 3.49 (t, 2H, J = 6.75 Hz), 5.46 (t, 2H, J = 7.63 Hz), 8.08 (m, 2H), 8.32 (t, 1H, J = 7.63, 8.22 Hz), 8.50 (d, 1H, J = 8.51 Hz), 8.72 (d, 1H, J = 8.8 Hz) and 10.08 (d, 1H, J = 5.87 Hz).

1'-(3'-Iodopropyl)-3-methyl-oxa-4'-cyanine iodide, 1

Triethylamine (0.65 ml, 4.71 mmol) was added drop wise to a solution of 2-methylthio-N-methylbenzoxazolium triflate (0.78 g, 2.37 mmol) in dichloromethane (30 ml) with stirring at room temperature. The reaction was mixed thoroughly for a few minutes and then lepidine iodopropiodide (1.0 g, 2.27 mmol) was slowly added to the mixture. Stirring was continued overnight at ambient temperature, which gradually produced an orange precipitate. Diethyl ether (100 ml) was added to the mixture and refrigerated overnight to precipitate all the product out of solution. The crude product was isolated by filtration, washed with cold 1:4 methanol/diethyl ether (2 x 50 ml), and recrystallised with methanol to give red/orange needles (**1**, 0.41 g, 30%): mp 225-227°C; ^{1}H-NMR (300 MHz, d-DMSO) ∂2.37 (t, 2H, J = 7), 3.32 (t, 2H, J = 7), 3.87 (s, 3H), 4.60 (t, 2H, J = 6Hz), 6.28 (s, 1H), 7.39 (t, 1H, J = 7), 7.48 (t, 1H, J = 8), 7.64 (d, 1H, J = 7), 7.73 (t, 1H, J = 8), 7.81 (d, 1H, J = 8), 7.93 (d, 2H, J = 7), 7.98 (t, 1H, J = 8), 8.10 (d, 1H, J = 9), 8.44 (d, 1H, J = 7) and 8.79 (d, 1H, J = 9).

2-(4-Iodophenyl)ethanol, 2

Lithium aluminum hydride (1M solution in diethyl ether, 7.6 ml, 7.6 mmol) was added drop wise to a solution of *p*-iodophenyl acetic acid (1.0 g, 3.8 mmol) in diethyl ether (20 ml) at 0°C. After complete addition, the suspension was maintained at 0°C for 30 minutes and then allowed to warm to room temperature. After 4 hours the reaction was cooled to 0°C and saturated aqueous magnesium sulfate added slowly (10ml). The aqueous layer was separated and extracted with diethyl ether (3 x 10ml). The combined organics were washed sequentially with 0.1N sodium hydroxide solution (3 x 10ml) and water (10 ml). The organics were then dried and solvent removed under reduced pressure, to give *p*-iodophenethanol, **2** as a colorless oil (0.91 g, 96 % yield), which was used without need for further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) ∂ 2.80 (t, 1H, J = 6), 2.87 (t, 1H, J = 6), 3.80 (t, 2H, J = 7), 6.98, (d, 1H, J = 8), 7.21 (t, 1H, J = 8), 7.32 (d, 1H, J = 6) and 7.63 (d, 1H, J = 8).

4-Iodophenethyl 4-methylbenzenesulfonate
Triethylamine (0.41 ml, 2.94 mmol) was added drop wise to a solution *p*-iodophenethanol, **2** (0.5g, 2.02mmol) in dichloromethane (5 ml) at 0°C. The reaction was maintained at 0°C for 15 minutes then *p*-toluenesulfonyl chloride (0.424 g, 2.22 mmol) was added in small portions. After a further 15 minutes at 0°C the reaction was allowed to warm to room temperature and stir overnight. Dichloromethane (20 ml) and water (20 ml) were added and the organic layer separated washed with brine, dried and solvent removed under reduced pressure. Chromatography of the residue with 5% ethyl acetate/hexane gave 4-Iodophenethyl 4-methylbenzenesulfonate as a colorless oil (0.66 g, 81 % yield). ^{1}H-NMR (300 MHz, $CDCl_3$) ∂ 2.42 (s, 3H), 2.86 (t, 1H, J = 7), 2.94 (t, 1H, J = 7), 4.19 (app. q, 2H, J = 7), 6.82, (d, 1H, J = 8), 7.07-7.12 (m, 1H), 7.22-7.27 (m, 3H),7.52 (d, 1H, J = 8), 7.62 (d, 1H, J = 8) and 7.67 (d, 1H, J = 8).

2-(4-Iodophenyl)-N,N-dimethylethanamine, 3
A mixture of 4-Iodophenethyl 4-methylbenzenesulfonate (0.5 g, 1.24 mmol) and dimethylamine (2 M solution in methanol, 2.49 ml, 4.98 mmol) were sealed in a small pressure reaction vessel and heated to 60°C. After 4 hours the reaction vessel was cooled to 0°C and opened then the solvent removed under reduced pressure. Chromatography of the residue in 5% methanol/chloroform gave 4-Iodo-N,N-dimethylphenethylamine, **3** (0.236 g, 69% yield) as a colorless oil. ^{1}H-nmr (400 MHz, CD_3Cl) ∂ 2.27 (s, 6H), 2.49 (t, 2H, J = 9), 2.71 (t, 2H, J = 7), 6.95 (d, 2H, J = 8) and 7.58 (d, 2H, J = 8).

4-Bromo-N,N-dimethylphenethylamine, 4
Sodiumcyanoborohydride (3.0 g, 48 mmol) was added to a solution of 4-bromophenethylamine (1.5 ml, 9.7 mmol) in aqueous formaldehyde (37%, 4.0 ml, 49 mmol). After 20 min., acetic acid was added to maintain pH 7. The reaction was stirred for a further hour and the solvent removed under vacuum. The residue was diluted with ether (20 ml) and washed with aqueous potassium hydroxide (3N, 3 x 10 ml). The organic layer was extracted with hydrochloric acid (1N, 3 x 20 ml) and the combined acid washes were neutralized with saturated aqueous potassium hydroxide. The aqueous solution was then extracted with diethyl ether (3 x 20 ml) dried and the solvent removed under reduced pressure. Chromatography of the residue with 5% methanol/chloroform gave amine, **4** as a colorless oil (1.84 g, 83% yield). ^{1}H-nmr (400 MHz, CD_3Cl) 2.27 (s, 6H, 2.49 (t, 2H, J = 6), 2.71 (t, 2H, J = 6), 7.06 (d, 2H, J = 8) and 7.38 (d, 2H, J = 8).

1-(dimethylamino)-3-fluoropropan-2-ol, 8
Dimethylamine (1M in methanol, 6.57 ml, 13.14 mmol) was added to a solution of epifluorohydrin (0.5 g, 6.57 mmol) in methanol (5 ml) at 0°C in a pressure reaction vessel. The reaction vessel was flushed with argon, sealed and heated to 60°C for 4 hours. Upon cooling the solvent was removed under reduced pressure. The residue was taken up in diethyl ether (15 ml) and hydrogen chloride in diethyl ether (1M, 15 ml, 15 mmol) was carefully added at 0 °C. The white hydrochloride salt was separated and washed with ether (2 x 5 ml) then dried under vacuum. Saturated aqueous sodium bicarbonate (10 ml) was added to the hydrochloride salt to form the free amine and the solution extracted with chloroform (3 x 10 ml). The combined organic extracts were dried and the solvent removed to give 1-(dimethylamino)-3-fluoropropan-2-ol, **8** as a colorless

oil (0.71 g, 89% yield), which was used without need for further purification. ^{1}H-nmr (400 MHz, CD_3Cl) ∂ 2.22-2.28 (m, 1H), 2.29 (s, 6H), 2.49 (t, 1H, J = 13), 3.80-3.97 (m, 1H) and 4.24-4.56 (m, 2H).

1'-(3'-Dimethylphenethylammoniumpropyl)-3-methyl-oxa-4'-cyanine diiodide, 5
N,N-Dimethylphenethylamine (29.5 µl, 0.176 mmol) was added into a suspension of 1'-(3'-iodopropyl)-3-methyl-oxa-4'-cyanine iodide, 1 (0.05 g, 0.088 mmol) in methanol (0.5 ml) at ambient temperature in a conical vial equipped with a spin vain. The reaction vessel was flushed with argon, sealed and heated to 90 °C, during which time the suspension became an orange solution. After 14 hours the reaction was slowly cooled to room temperature and the cyanine 5 precipitated by the addition of diethyl ether (4 ml). The crude cyanine was purified by recrystallisation from methanol (0.054 g, 85%). Mp 175-180°C; ^{1}H-nmr (400 MHz, d-DMSO) ∂2.98-3.08 (m, 2H), 3.12 (s, 6H), 3.31 (d, 2H, J = 7), 3.45-3.62 (m, 4H), 3.88 (s, 3H), 4.58-4.68 (m, 2H), 6.32 (s, 1H), 7.23-7.37 (m, 5H), 7.38-7.54 (m, 2H), 7.67 (d, 1H, J = 8), 7.75 (t, 1H, J = 8), 7.80 (t, 1H, J = 8), 7.96 (d, 1H, J=7), 8.01 (t, 1H, J = 7), 8.19 (d, 1H, J = 8), 8.48 (d, 1H, J = 7) and 8.80 (d, 1H, J = 8); HRMS m/z calcd for $C_{31}H_{35}IN_3O^+$ 592.18193, found 592.18030.

1'-(3'-Dimethyl-4-bromophenethylammoniumpropyl)-3-methyl-oxa-4'-cyanine diiodide, 6
The general procedure described above was used to prepare cyanine **5**, using 1'-(3'-iodopropyl)-3-methyl-oxa-4'-cyanine iodide, **1** (0.05 g, 0.088 mmol), amine **4** (0.04 g, 0.175 mmol) in methanol (0.5 ml). The reaction gave cyanine **6**, which was purified by recrystallisation from methanol (0.051 g, 73%). Mp 166-170°C; ^{1}H-nmr (400 MHz, d-DMSO) ∂2.99-3.07 (m, 2H), 3.11 (s, 6H), 3.30 (d, 2H, J = 7), 3.43-3.62 (m, 4H), 3.87 (s, 3H), 4.62 (t, 2H, J = 7), 6.30 (s, 1H), 7.26 (d, 2H, J = 8), 7.39 (t, 1H, J = 7), 7.49 (t, 1H, J = 8), 7.54 (d, 2H, J = 8), 7.65 (d, 1H, J = 8), 7.74 (t, 1H, J = 8), 7.81 (d, 1H, J = 8), 7.95 (d, 1H, J = 7), 8.00 (t, 1H, J = 7), 8.19 (d, 1H, J = 8), 8.48 (d, 1H, J = 7) and 8.79 (d, 1H, J = 8); HRMS m/z calcd for $C_{31}H_{34}BrIN_3O^+$ 670.09244, found 670.09170.

1'-(3'-Dimethyl-4-iodophenethylammoniumpropyl)-3-methyl-oxa-4'-cyanine diiodide, 7
The general procedure described above was used to prepare cyanine **5**, using 1'-(3'-iodopropyl)-3-methyl-oxa-4'-cyanine iodide, **1** (0.05 g, 0.088 mmol), amine **3** (0.48 g, 0.176 mmol) in methanol (0.5 ml). The reaction gave cyanine **7**, which was purified by recrystallisation from methanol (0.049 g, 66%). Mp 188-190°C; ^{1}H-nmr (400 MHz, d-DMSO) ∂ 2.98-3.08 (m, 2H), 3.10 (s, 6H), 3.30 (d, 2H, J = 7), 3.42-3.62 (m, 4H), 3.87 (s, 3H), 4.54-4.69 (m, 2H), 6.31 (s, 1H), 7.10 (d, 2H, J = 8), 7.42 (t, 1H, J = 8), 7.49 (t, 1H, J = 8), 7.64-7.74 (m, 4H), 7.78 (t, 1H, J = 8), 7.94 (d, 1H, J = 7), 8.00 (t, 1H, J = 7), 8.17 (d, 1H, J = 8) and 8.45 (d, 1H, J = 7), 8.79 (d, 1H, J = 8); HRMS m/z calcd for $C_{31}H_{34}I_2N_3O^+$ 718.07858, found 718.07610.

1'-(3'-Dimethyl-3-fluoro-2-propanol)-3-methyl-oxa-4'-cyanine diiodide, 9
The general procedure described above was used to prepare cyanine **5**, using 1'-(3'-iodopropyl)-3-methyl-oxa-4'-cyanine iodide, **1** (0.05 g, 0.088 mmol), amine **8** (0.021 g, 0.176 mmol) in methanol (0.5 ml). The reaction gave cyanine **9**, which was purified by

recrystallisation from methanol (0.047 g, 77%). Mp 208-212°C; ^{1}H-nmr (400 MHz, d-DMSO) ∂3.10 (s, 3H), 3.11 (s, 3H), 3.28-3.34 (m, 2H), 3.37-3.49 (m, 2H), 3.57 (t, 2H, J = 7), 3.87 (s, 3H), 4.25-4.46 (m, 3H, J_{HF} = 45), 4.59, (t, 2H, J = 7), 6.31 (s, 1H), 7.39 (t, 1H, J = 8), 7.49 (t, 1H, J = 8), 7.70 (d, 1H, J = 8), 7.73 (t, 1H, J = 8), 7.80 (d, 1H, J = 8), 7.94 (d, 1H, J = 7), 8.00 (d, 1H, J = 7), 8.14 (d, 1H, J = 8), 8.44 (d, 1H, J = 7) and 8.79 (d, 1H, J = 8); HRMS m/z calcd for $C_{26}H_{32}FIN_3O_2^+$ 564.15020, found 564.15177.

Biological

All biological experiments were performed using stock solutions of analogs at a concentration of 1 mM in DMSA.

DNA-binding experiments were performed with a stock solution of calf thymus DNA (Sigma) at 1 mg/6 ml water. Spectral measurements were made on solutions of analogs diluted to 1 μM final concentration with either the DNA stock solution or water. Absorbance measurements were determined over a 200-700 nm wavelength range, utilizing a Perkin-Elmer Lambda 3 uv/vis scanning spectrophotometer. Once the absorbance maxima were determined for each analog in the presence and absence of DNA, these wavelengths were used as excitation wavelengths in a Noriba Fluorolog spectrofluorimeter to measure emission spectra and determine emission maximal wavelengths.

Cellular uptake was determined using mink lung epithelial cells plated in T75 tissue culture flasks and grown in Dubelcco's modified Eagle's medium supplemented with 10% fetal bovine serum under 5% CO_2 at 37 C. After 3 days, apoptosis was induced in half of the dishes via exposure to X-rays (5 Gy) using a 320KV/20m PANTAK X-RAY source. Control cells were sham-irradiated. Twenty hours after exposure to the radiation, one of the four analogs or the commercial Yo-Pro dye was added to each dish at a final concentration of 1uM and incubated for 20 min at room temperature to allow for cellular uptake. Uptake was determined by the generation of a fluorescent signal, indicative of the analog binding to the cellular DNA. Fluorescent cells were counted under a Zeiss Axiovert fluorescent microscope (Ex/Em= 490 nm/525 nm). These experiments were repeated 3 times for each compound.

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